Attorney Docket No. 5405.239CT PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Williams
Serial No.: 10/625,134
Filed: July 23, 2003
For: *Use of polymorphism of the serotonin transporter gene promoter as a predictor of disease risk*

Group Art Unit: 1634
Examiner: J. Sitton
Confirmation No. 8271

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

January 5, 2007

Declaration of Redford Williams, M.D. Pursuant to 37 C.F.R. § 1.132

I, Redford Williams, do hereby declare and say as follows:

1. I am a named inventor on US Provisional Application No. 60/162, 390 (the '390 provisional application) and US Application No. 10/625,134 (the '134 application) and of the subject matter claimed therein.

2. I have a Medical Degree (M.D.) from Yale University School of Medicine, New Haven, CT. I am a Professor of Psychiatry and Medicine at Duke University in Durham, North Carolina. I have been conducting research in the area of psychosomatic medicine for 40 years and have authored or co-authored more than 200 publications related to this area.

3. This Declaration sets forth: I) the state of the art at the time of the present invention regarding an association between cardiovascular disease and an increased physiological response to psychological stress, wherein the response is increased blood pressure; II) data demonstrating that the association between the long allele genotype and an increased physiological response to psychological stress, wherein the response is increased blood pressure, is consistently significant across gender and ethnic groups; and III) documentation that studies by other scientific groups have substantiated the presently claimed invention

I. The following publications A-D, a copy of each of which was provided to the Examiner with the response filed March 31, 2006, demonstrate that at the time of the present invention, it was known in the art that there was an association between cardiovascular disease and an increased physiological response to psychological stress

wherein the response is increased blood pressure. Relevant portions of these papers are summarized below.

A) Rozanski et al. ("Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy" *Circulation* 99:2192-2217 (1999)) is a review article summarizing studies evaluating the effect of psychological stress on the pathogenesis of cardiovascular disease. In the section entitled "Sympathetic Nervous System Hyperresponsivity" (page 2207, second column through page 2208, first column), the term "cardiovascular reactivity" (as also used on page 5, lines 5-6 of the '390 provisional application) is defined as a "...dispositional tendency to exhibit exaggerated heart rate and blood pressure responses when encountering behavioral stimuli experienced as engaging, challenging or aversive." The authors of Rozanski et al. go on to state that "...it has been postulated that individuals manifesting more elevated heart rate and blood pressures to such psychological challenge (ie, "hot reactors") may experience more substantial sympathetic nervous system responses over time than "cold reactors" and that this may in turn promote the development of atherosclerosis." Various articles are then summarized that examine this hypothesis, with the conclusion that "[c]ombined, these recent animal and human studies raise the possibility that sympathetic hyperresponsivity may constitute a risk factor for the development or progression of CAD." This conclusion is also stated in the Abstract of this paper, which begins with the comment that "[r]ecent studies provide clear and convincing evidence that psychosocial factors contribute significantly to the pathogenesis and expression of coronary artery disease."

B) In Kamarck et al. ("Exaggerated blood pressure responses during mental stress are associated with enhanced carotid atherosclerosis in middle-aged Finnish men" *Circulation* 96:3842-3848 (1997)), "cardiovascular reactivity" is described as an exaggerated cardiovascular response to mental stress." (Introduction, first paragraph). The authors of Kamarck et al. state that theirs is the "first study to examine the association between standardized measures of cardiovascular reactivity and quantitative assessment of carotid atherosclerosis in a population sample" (Discussion, second paragraph) and conclude from their studies that they have shown "...that blood pressure reactivity to mental stress is positively associated with ultrasound measures of carotid artery wall thickness, a marker of atherosclerosis in a sample of middle-aged Finnish men." (Discussion, first paragraph). It is further concluded in Kamarck et al. that, on the basis of the studies described, "[t[he tendency to show exaggerated pressor responses to mental stress is a significant independent correlate of atherosclerosis in this population sample of Finnish men." (Abstract, last paragraph).

C) Matthews et al. ("Stress-induced pulse pressure change predicts women's carotid atherosclerosis" *Stroke* 29:1525-1530 (1999)) describes an evaluation of "...the association between stress-induced hemodynamic responses and carotid atherosclerosis in 254 postmenopausal women." (Abstract, first paragraph). The hemodynamic response measured was the magnitude in the change in blood pressure and heart rate during the performance of stressful tasks. In a follow up study of these

subjects about two years later, average intima-media thickness (IMT) and focal plaque in the common carotid artery, bulb and internal carotid artery were measured. These authors explain that IMT is thought to be an early marker of diffuse atherosclerosis and that high levels of IMT in asymptomatic men and women predict new clinical CHD and stroke and that therefore, "...ultrasound measures of carotid IMT and plaque can provide a test of the association of the cardiovascular risk associated with mental stress responses." (Introduction, second paragraph). These authors concluded from these studies that "...these findings suggest that a large pulse pressure response to mental stress is an important predictor of subsequent IMT." (Discussion, first paragraph).

D) In Everson et al. ("Stress-induced blood pressure reactivity and incident stroke in middle-aged men." *Stroke* 32:1263-1270 (2001)), studies are described wherein these authors examined whether stress-induced cardiovascular reactivity contributes directly to increased risk of stroke. For this study, an association between exaggerated blood pressure reactivity (i.e., cardiovascular reactivity) in response to anticipation of physical exercise (page 1265, first column, first full paragraph) with incident stroke was examined in 2303 men. The results of this study showed that those subjects with exaggerated systolic reactivity had a significantly greater risk of stroke than less reactive subjects (page 1265, second column, under heading "All Strokes") and these authors concluded that "[t]his study provides convincing evidence that exaggerated BP responses to stress contribute to increased risk of stroke and particularly stroke due to ischemia or thromboembolism in middle-aged and older men." (page 1266, second column, first paragraph of Discussion section).

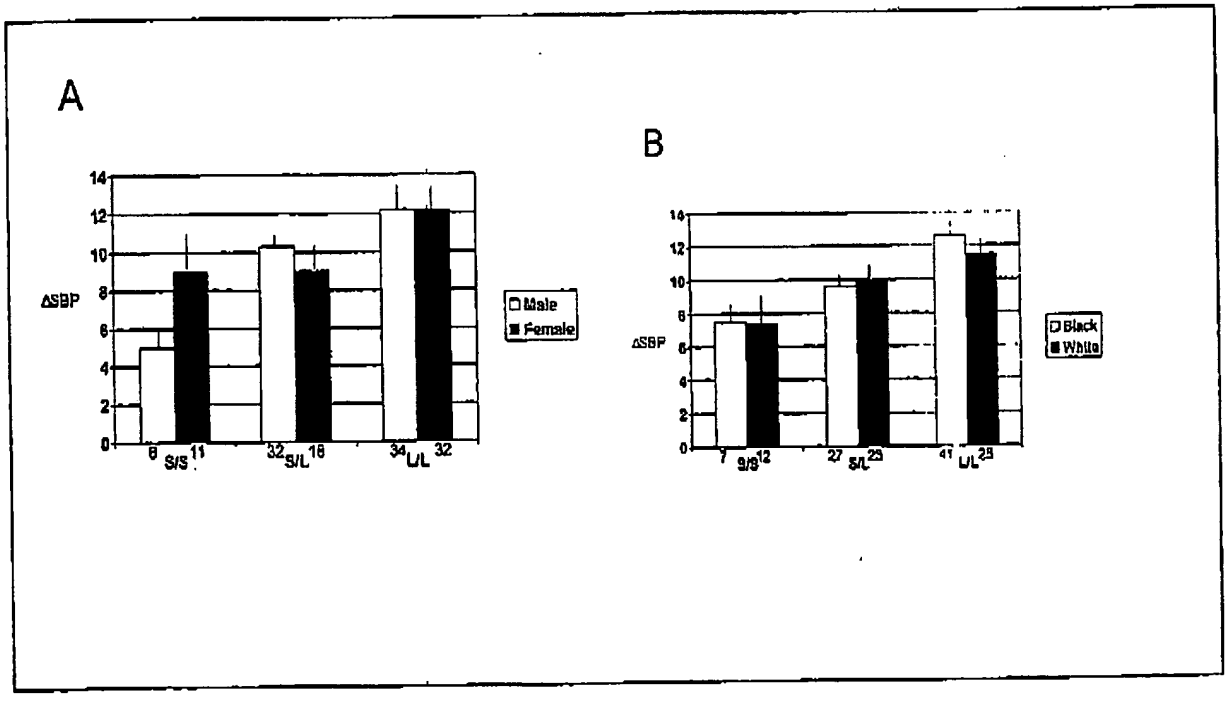
Although the publication date of this paper is after the filing date of my patent application, this paper discusses further aspects of the longitudinal study described in the Kamarck et al. 1997 paper reviewed above, wherein baseline data collected from subjects between 1984 and 1989 and follow up ascertainment of stroke in these subjects through 1997 (page 1265, first column under heading "Ascertainment of Strokes.") were analyzed. These authors indicate that they were the first to demonstrate a direct link between cardiovascular reactivity and increased risk of stroke, thereby supporting "...the growing literature on the importance of hemodynamic or cardiovascular reactivity in the development and progression of cardiovascular diseases." (page 1266, second column, first paragraph of Discussion).

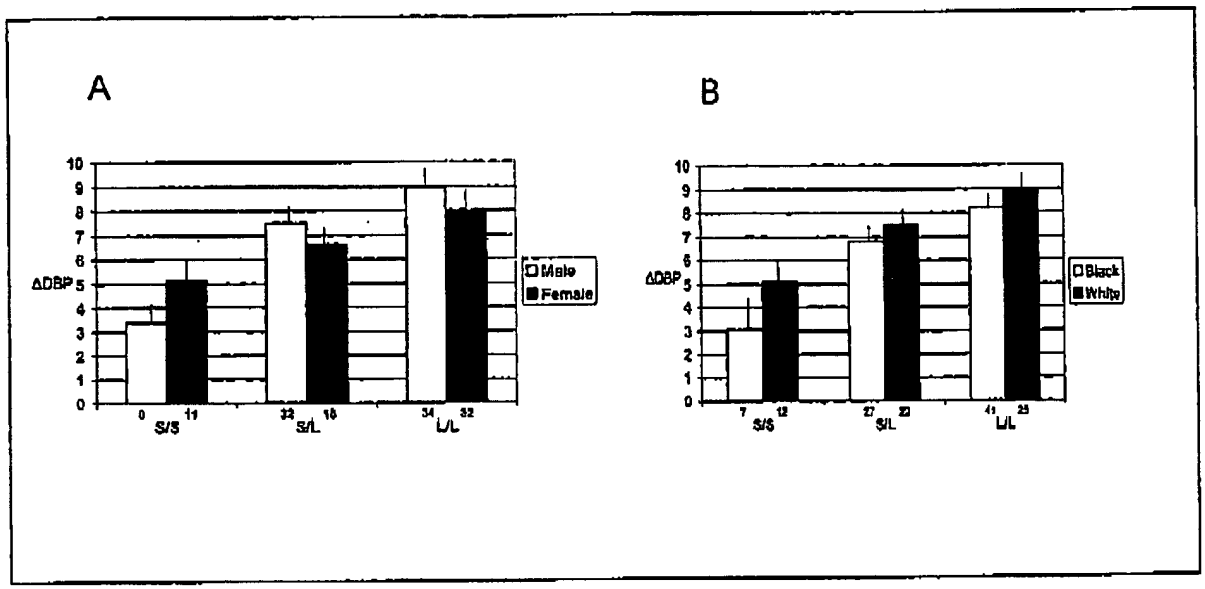
These publications exemplify the state of the art at the time of the present invention regarding an association between cardiovascular disease and an increased physiological response to psychological stress, wherein the response is increased blood pressure (i.e., cardiovascular reactivity).

II. The Williams et al. 2003 publication cited in the July 5, 2006 Office Action (*Neuropsychopharmacology* 28:533-541 (2003)) includes results of the same studies described in the '390 provisional application and in the '134 application, with an expansion of the sample size. Although the association between 5HTTLPR genotypes and levels of 5HIAA in CSF is stated in the Williams et al. 2003 paper to vary as a

function of gender and race, it was shown in the same subjects described therein that the association between 5HTTLPR genotypes and cardiovascular reactivity to psychological stress did not differ in men and women or in blacks and whites.

The figures below show data from the same sample of subjects described in the Williams et al. 2003 publication. Data in these figures demonstrate in both males and females (A) and in both blacks and whites (B) that carriers of the 5HTTLPR L allele (L/L or S/L) exhibited larger cardiovascular reactivity than those with the S/S genotype in response to the psychological stress protocols described in my patent applications and in Williams et al. (*Psychosom Med.* 63:300-305 (2001)). It is also stated in the Williams et al. 2001 publication that with statistical control for 5HTTLPR genotype, the CSF 5HIAA association with cardiovascular reactivity to psychological stress became nonsignificant, but that with control for CSF 5HIAA, the 5HTTLPR genotype association with cardiovascular reactivity to psychological stress remained significant (page 302, second column, last paragraph).





III. Publications subsequent to the filing of my patent applications by two other scientific groups provide data that demonstrate that the presence of the 5HTTLPR L allele is associated with cardiovascular disease and therefore will identify a subject having an increased risk of developing cardiovascular disease, thereby substantiating my invention.

Specifically, studies by Fumeron et al. (*Circulation* 105:2943-2945 (2002)) and Coto et al. (*Clin Sci.* 104:241-245 (2003)) (a copy of each of which is enclosed) have shown that the 5HTTLPR L allele is associated with increased risk of myocardial infarction. These studies provide independent replications of an association between the 5HTTLPR L allele and increased risk of cardiovascular disease, as demonstrated with myocardial infarction. Thus, these groups corroborate my invention, in which I have established an association between the 5HTTLPR L allele and increased cardiovascular reactivity to psychological stress and the known association between increased cardiovascular reactivity to psychological stress and increased cardiovascular disease risk and the subsequent methodology of using the 5HTTLPR L allele as a marker to identify subjects who are at increased risk of developing cardiovascular disease. This corroboration is further supported by the fact that Fumeron et al. cites my findings in the Williams et al. 2001 publication of increased cardiovascular reactivity in response to stress in people with the L allele as a mechanism that could account for their finding of an association between the 5HTTLPR L allele and increased myocardial infarction risk (page 2945, first column, first paragraph).

4.I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Redford Williams

Redford Williams, M.D.

5 January 2007

Date